Supplement to the Journal of Nematology 30(4S):547–552. 1998.

Possible Transfer of Resistance to *Heterodera glycines* from *Glycine tomentella* to *Glycine max*[1]

R. D. Riggs,[2] S. Wang,[2] R. J. Singh,[3] and T. Hymowitz[3]

Abstract: Eight wild perennial *Glycine* species (*G. argyrea, G. canescens, G. curvata, G. cyrtoloba, G. latifolia, G. microphylla, G. tabacina,* and *G. tomentella*) were evaluated for resistance to isolates of races 1, 3, and 14 of *Heterodera glycines.* In a second experiment, reproduction of isolates of races 3, 5, and 14 of *H. glycines* on five of the wild perennial species was determined. Seventy-one derived fertile lines ($2n = 40$) that were hybrids between *G. max* cv Clark 63 and *G. tomentella* also were evaluated for resistance to isolates of races 3, 5, and 14. All of the wild perennial *Glycine* species were resistant (Female Indices [FI] less than 10) to all of the isolates that were tested on them. In most cases no females matured. The soybean cvs. Clark 63 and Altona, which were tested at the same time as the hybrids, were susceptible to all isolates of *H. glycines* tested. When the tests were combined and a single FI calculated with the average number of females on Lee 74, one derived fertile line was resistant to race 3, three derived fertile lines were resistant to race 5, and five derived fertile lines were resistant to race 14. Thus, transfer of resistance to *H. glycines* from *G. tomentella* to *G. max* apparently occurred.

Key words: Glycine species, *Heterodera glycines,* interspecific hybrids, nematode, resistance, soybean, soybean cyst nematode.

The soybean cyst nematode, *Heterodera glycines* Ichinohe, is a serious pest on soybean, *Glycine max* (L.) Merr., in most countries where the crop is grown (Noel, 1992). Nematicides have been used to reduce damage by *H. glycines* (Schmitt et al., 1983), but some have been eliminated because of a danger to users (Johnson and Feldmesser, 1987) whereas others provide inconsistent control or do not provide an economic return. Crop/cultivar rotations are recommended for the management of *H. glycines* population levels but are not always used by the growers (Wrather et al., 1992). Soybean cultivars with resistance to *H. glycines* should be an integral part of management programs. However, in many instances, resistant cultivars have been grown continuously, which has resulted in the selection of *H. glycines* populations that reproduce on the resistant cultivars (Young et al., 1986). To date, new sources of resistance have been found each time the resistance from an old source has been broken (Caviness, 1992).

The soybean cultivar Peking was the source of resistance for the first yellow-seeded *H. glycines*-resistant cultivar (Pickett) released for grower use (Caldwell et al., 1960). Cultivars derived from Peking generally were resistant to races 1 and 3 but susceptible to race 14. Later, PI88788 was used as a source of resistance to race 14, and, finally, PI437654 was used as the source of resistance to all known races of *H. glycines.*

Glycine (subgenus *Glycine*) contains 16 wild perennial species (Kollipara et al., 1997), all indigenous to Australia and grown in diverse geographical areas under a wide range of climatic conditions. These *Glycine* spp. had not been screened for resistance to *H. glycines.* The objectives of these experiments were to determine the susceptibility of the wild *Glycine* species to *H. glycines* and whether resistance to *H. glycines* was transferred to hybrids of *G. tomentella* and *G. max.*

Materials and Methods

Plant material: Seeds of *Glycine arenaria* Tind., *G. argyrea* Tind., *G. canescens* F.J. Herm., *G. curvata* Tind., *G. cyrtoloba* Tind., *G. falcata* Benth., *G. latifolia* (Benth.) Newell & Hymowitz, *G. microphylla* (Benth.) Tind., *G. tabacina* (Labill.) Benth., and *G. tomentella* were provided by T. Hymowitz from Univer-

Received for publication 21 October 1997.
[1] Approved for publication by the Director of the Arkansas Agricultural Experiment Station. This research was supported by funds from the soybean growers in Arkansas through a grant from the Arkansas Soybean Promotion Board.
[2] Department of Plant Pathology, University of Arkansas, Fayetteville, AR 72701. Second author is a visiting scientist from Beijing Agricultural University, Beijing 100094, PRC.
[3] Department of Crop Sciences, University of Illinois, 1102 South Goodwin Ave., Urbana, IL 61801.
E-mail: rdriggs@comp.uark.edu

sity of Illinois, Urbana, Illinois. Seeds of derived fertile lines from crosses between *G. max* cv. Altona ($2n = 40$) and *G. tomentella* PI 483,218 ($2n = 78$), which had been backcrossed to cv. Clark 63 ($2n = 40$) four times, were provided by R. J. Singh and T. Hymowitz.

Seeds were germinated in vermiculite and transplanted as seedlings in the cotyledon stage into fine sandy soil in 7.5-cm-diam. (for the first *Glycine* spp. test) or 10-cm-diam. (for the tests of the interspecific crosses) clay pots before nematodes were added.

Nematode populations: Greenhouse stock culture isolates of *H. glycines* were produced as follows: race 1 was cultured on soybean cv. Lee 74 and PI 88,788 growing in the same pots; race 3 on cv. Lee 74; race 5 on cv. Bedford; and race 14 on cv. Pickett. Females and (or) cysts (hereafter called cysts) were rubbed from the roots into water, stirred, and poured through nested 850-µm-pore (20-mesh) and 250-µm-pore (60-mesh) sieves. The cysts caught on the 250-µm-pore sieve were washed into a ground glass homogenizer and broken to release the eggs. The resulting suspension was poured through nested 75-µm-pore and 25-µm-pore (500-mesh) sieves, and eggs were collected on the 25-µm-pore sieve for tests. Approximately 4,000 eggs and second-stage juveniles of *H. glycines* were added to the fine sandy soil in which each test plant was growing. Lee 74 and the *H. glycines* race determination soybean lines (Golden et al., 1970; Riggs and Schmitt, 1988) were included in the tests to verify the races of the isolates of *H. glycines* used. Three to five replications of each plant were used depending on the number of seeds that germinated.

Plant susceptibility determination: Five weeks after nematode eggs were added to the soil, newly produced cysts were extracted by the same procedure used to obtain test nematodes. The females extracted from each plant were counted at ×10 magnification with a stereoscopic microscope. The tests were repeated once.

The numbers of cysts on each *H. glycines* race differential and on each test plant were converted to a Female Index (FI), a percentage of the number of cysts on the soybean cv. Lee 74. Plants with a mean FI less than 10 were considered to be resistant to a particular race, those with a mean FI of 10 to 30 were considered moderately resistant, those with a mean FI of 31 to 60 were considered moderately susceptible, and those with a

TABLE 1. Numbers of females of *Heterodera glycines* per pot of *Glycine* spp. inoculated with race 1, 3, or 14 of *H. glycines*.

		Females per pot and FI[a]					
		Race 1		Race 2		Race 14	
Glycine sp.	Cultivar or PI number	Mean	FI (%)	Mean	FI (%)	Mean	FI (%)
G. max	Lee 74	250	100.0	166	100.0	783	100.0
G. max	Pickett	2	0.6	0	0.1	929	118.6
G. max	Peking	0	0.0	0	0.1	354	45.3
G. max	PI 88,788	42	16.7	1	0.7	19	2.4
G. max	PI 90,763	1	0.2	0	0.0	290	37.1
G. max	Hartwig	0	0.0	0	0.0	0	0.2
G. argyrea	PI 505,151	0	0.0	1	0.4	2	0.3
C. canescens	PI 440,928	11	4.3	7	4.2	1	0.1
G. curvata	PI 505,166	0	0.0	0	0.0	1	0.1
G. cyrtoloba	PI 440,963	0	0.0	0	0.0	0	0.0
G latifolia	PI 378,709	—[c]	—	0	0.0	2	0.3
G. microphylla	PI 440,956	1	0.3	4	2.6	0	0.0
G. tabacina[b]	PI 440,956	30	12.1	0	0.2	1	0.1
G. tabacina[b]	PI 483,204	0	0.0	0	0.2	0	0.0
G. tomentella[b]	PI 441,005	0	0.1	0	0.0	0	0.0

[a] FI = Female Index, the number of cysts on line per number of cysts on Lee × 100.
[b] $2n = 80$, other accessions $2n = 40$.
[c] Dash indicates no test.

TABLE 2. Numbers of females of *Heterodera glycines* per pot of *Glycine* spp. and their hybrids inoculated with race 3 of *H. glycines*.

Glycine sp. or hybrid	Cultivar or PI number	Females per pot and FI[a]					
		Test 1		Test 2		Test mean	
		Mean	FI (%)	Mean	FI (%)	Cysts	FI (%)
G. max	Lee 74	246	100.0	779	100.0	513	100.0
G. max	Pickett	2	0.8	4	0.5	3	0.6
G. max	Peking	0	0.0	0	0.0	0	0.0
G. max	PI 88,788	3	1.4	0	0.04	1	0.2
G. max	PI 90,763	4	1.6	1	0.2	3	0.5
G. arenaria	PI 505,204	0	0.0	—[d]	—	0	0.0
G. argyrea	PI 505,151	—	—	0	0.0	0	0.0
G. cyrtoloba	PI 440,963	—	—	0	0.0	0	0.0
G. microphylla	PI 440,956	0	0.0	—	—	0	0.0
G. tabacina[b]	PI 440,996	—	—	0	0.0	0	0.0
G. tabacina[b]	PI 483,202	—	—	0	0.0	0	0.0
G. tomentella[c]	PI 483,218	0	0.0	0	0.0	0	0.0
G. max	Altona	138	56.1	580	74.4	460	89.8
G. max	Clark 63	198	80.5	468	59.6	388	75.7
H 706-1		72	29.3	393	50.4	308	60.1
H 719-1		—	—	192	24.6	192	24.6
H 720-1		—	—	108	13.9	108	13.9
H 722-4		72	29.3	472	60.6	372	72.6
H 722-5		66	26.8	576	73.9	465	90.7
H 722-6		0	0.0	344	44.2	258	50.3
H 722-7		—	—	450	57.8	450	57.8
H 722-9		36	14.6	—	—	36	14.6
H 724-1		4	1.4	531	68.2	320	62.4
H 725-1		72	29.3	358	46.0	254	49.6
H 725-3		57	23.2	126	16.2	98	19.2
H 729-1		66	26.8	174	22.3	120	23.4
H 733-1		1	0.4	210	27.0	158	30.8
H 736-1		21	8.5	—	—	21	8.5
H 736-3		48	19.5	210	27.0	156	30.4
H 745-2		51	20.7	336	43.1	194	37.8
H 749-1		58	23.7	564	72.4	408	79.6
H 762-1		72	29.3	1892	114.5	678	134.0
H 765-1		51	20.7	438	56.2	341	66.5
H 765-3		62	25.2	1780	100.1	421	82.1
H 766-2		180	73.2	519	66.7	350	68.2
H 788-1		51	20.7	394	50.6	257	50.1
H 789-1		54	22.0	456	58.5	356	69.4
H 810-1		190	77.2	190	24.4	190	37.1
MT-02-06		116	47.2	194	24.9	155	30.2
MT-03-03		108	43.9	151	19.4	140	27.4
MT-05-08		48	19.5	414	53.1	231	45.1
MT-05-10		66	26.8	—	—	66	26.8

[a] FI = Female Index, the number of cysts on line per number of cysts on Lee × 100.
[b] $2n = 80$.
[c] $2n = 78$, other accessions $2n = 40$.
[d] Dash indicates no test.

mean FI greater than 60 were considered susceptible (Schmitt and Shannon, 1992).

RESULTS

Screening of wild perennial species: Reproduction levels of races 1, 3, and 14 varied on the eight *Glycine* spp. tested (Table 1). Race 1 did not mature on *G. argyrea, G. curvata, G. cyrtoloba,* or *G. tabacina;* race 3 did not mature on *G. curvata, G. cyrtoloba, G. latifolia,* or *G. tomentella;* and race 14 did not mature on *G. crytoloba, G. microphylla,* or *G. tomentella.* Although some females matured on all other *Glycine* species, so few were found that all were considered to be resistant (FI < 10) except that *G. tabacina* PI 440956 was only moderately resistant to race 1 (Table 1). In a second test of the resistance of *G. argyrea, G. cyrtoloba, G. tabacina,* and *G. tomentella* to races 3, 5, and 14 only a few cysts of race 14 developed and only on *G. argyrea* and *G. tomentella* (Tables 2–4).

Screening of derived fertile lines of soybean × G. tomentella: Of the 70 lines tested for resistance to race 3, three were moderately resistant, and none was resistant in both tests; four were moderately resistant, and one was resistant in one test, but they were not used in the other test (Table 2). In addition, 16 hybrids were moderately susceptible, and four were susceptible in both tests with race 3 (data not shown). When the two tests were averaged together, one hybrid was resistant, nine were moderately resistant, 35 were moderately susceptible, and 25 were susceptible.

Of the 66 hybrid lines tested for resistance to race 5, none were resistant or moderately resistant in both tests (Table 3). No hybrids were moderately susceptible in both tests; 36 were susceptible (data not shown). Two hybrids were resistant and one moderately resistant in one test, but they were not tested in the other. When the two tests were averaged together, two hybrids were resistant, four were moderately resistant, five were moderately susceptible, and 55 were susceptible.

Sixty-five hybrid lines were tested for resistance to race 14, and none were resistant or moderately resistant in both tests; four that were resistant and five that were moderately resistant in one test were not included in the other test (Table 4). When the results of the two tests were combined, 21 hybrids were moderately susceptible and 33 were susceptible (results not shown).

Of the 10 lines that were resistant to at

TABLE 3. Numbers of females of *Heterodera glycines* per pot of *Glycine* spp. and their hybrids inoculated with race 5 of *H. glycines.*

Glycine sp. or hybrid	Cultivar or PI number	Number of females of *H. glycines* per pot and FI[a]					
		Test 1		Test 2		Test mean	
		Mean	FI (%)	Mean	FI (%)	Cysts	FI (%)
G. max	Lee 74	252	100.0	318	100.0	285	100.0
G. max	Pickett	8	3.0	152	47.8	80	28.0
G. max	Peking	1	0.4	2	0.7	2	0.7
G. max	PI 88,788	142	56.3	162	50.9	150	52.6
G. max	PI 90,763	—[c]	—	0	0.0	0	0.0
G. arenaria	PI 505,204	0	0.0	—	—	0	0.0
G. argyrea	PI 505,121	—	—	0	0.0	0	0.0
G. microphylla	PI 440,956	0	0.0	—	—	0	0.0
G. tabacina[b]	PI 483,202	—	—	0	0.0	0	0.0
G. tomentella[b]	PI 446,993	0	0.0	0	0.0	0	0.0
G. tomentella[b]	PI 441,005	—	—	0	0.0	0	0.0
G. max	Altona	510	202.4	416	130.8	510	202.4
G. max	Clark 63	318	126.2	322	101.3	296	103.9
H 706-1		36	14.3	—	—	36	14.3
H 720-2		152	60.3	22	6.8	65	22.8
H 736-1		1	0.4	—	—	1	0.4
H 745-4		16	6.2	210	66.0	80	28.1
H 745-5		12	4.8	576	181.1	582	204.2
H 753-1		0	0.0	—	—	0	0.0
H 763-1		42	16.7	468	147.2	326	114.4
H 778-2		14	5.7	226	71.1	120	42.1
H 781-1		52	20.6	360	113.2	206	72.3
H 782-2		70	27.8	150	47.2	11	38.5
H 804-1		64	25.4	866	272.3	465	163.2
MT-05-18		72	28.6	4	1.3	27	9.5

[a] FI = Female Index, the number of cysts on line per number of cysts on Lee × 100.
[b] $2n$ = 80, other accessions $2n$ = 40.
[d] Dash indicates no test.

TABLE 4. Numbers of females of *Heterodera glycines* per pot of *Glycine* spp. and their hybrids inoculated with race 14 of *H. glycines*.

Glycine sp. or hybrid	Cultivar or PI number	Number of females of *H. glycines* per pot and FI[a]					
		Test 1		Test 2		Test mean	
		Mean	FI (%)	Mean	FI (%)	Cysts	FI (%)
G. max	Lee 74	305	99.9	490	100.0	397	100.0
G. max	Pickett	188	61.6	332	67.8	260	65.5
G. max	Peking	56	18.3	128	26.1	92	23.2
G. max	PI 88,788	3	1.1	19	3.8	11	2.8
G. max	PI 90,763	92	30.3	130	26.5	111	28.0
G. argyrea	PI 505,121	—[d]	—	1	0.3	1	0.3
G. microphylla	PI 440,956	0	0.0	—	—	0	0.0
G. tabacina[b]	PI 483,202	—	—	0	0.0	0	0.0
G. tomentella[b]	PI 446,993	1	0.2	—	—	1	0.2
G. tomentella[c]	PI 339,657	2	0.7	—	—	2	0.7
G. tomentella[b]	PI 441,005	—	—	1	0.2	1	0.2
G. max	Altona	189	62.0	618	126.1	444	111.8
G. max	Clark 63	282	92.6	153	31.2	271	68.3
H 722-5		0	0.0	—	—	0	0.0
H 725-1		40	13.0	—	—	40	10.1
H 733-1		81	26.6	—	—	80	20.2
H 745-5		3	1.0	—	—	3	0.8
H 755		6	2.0	318	64.9	162	40.8
H 763-1		2	0.7	—	—	2	0.5
H 765-1		30	9.7	—	—	30	7.6
H 782-2		60	19.8	824	168.2	442	111.3
H 784-1		318	104.3	128	26.1	223	56.2
H 788-1		52	17.0	168	34.3	110	27.7
H 804-1		142	46.6	128	26.1	135	34.0
H 805-2		30	9.8	340	69.4	222	55.9
H 814-1		436	143.0	86	17.6	261	65.7
MT-05-0		54	17.7	—	—	54	17.7
MT-05-1		48	15.7	—	—	48	15.7
MT-05-2		18	5.9	—	—	18	5.9

[a] FI = Female Index, the number of cysts on line per number of cysts on Lee × 100.
[b] $2n = 80$, other accessions $2n = 40$.
[c] $2n = 78$.
[d] Dash indicates not tested.

least one race, only one (H736-1) was resistant to two races, and it was moderately susceptible to race 14. One line (MT-05-18) was resistant to race 5 and moderately resistant to race 14 but susceptible to race 3. The other eight lines were either susceptible or moderately susceptible to the other two races.

DISCUSSION

Although all of the wild perennial *Glycine* species tested were resistant or moderately resistant to *H. glycines*, that does not mean they are automatically sources of resistance that are usable. Those that have 2n = 40 chromosomes would appear to be good candidates for hybridization with *G. max*, which has the same number. However, they may be completely incompatible with *G. max*. Even if they will hybridize, the progeny may not be very similar to *G. max*. *G. tomentella* (2n = 78, 80) and *G. tabacina* (2n = 80) would appear to be less likely candidates for hybridization because of the differences in chromosome numbers. In spite of the differences, fertile hybrids have been obtained between *G. max* and *G. tomentella* (Singh et al., 1993).

When hybridization occurs between species with different chromosome complements, inheritance patterns may not be normal. Pairings will not be between homologous chromosomes, and some chromosomes may not pair at all. A single recessive gene may be expressed because there is no dominant gene at the same locus to prevent its expression. In the backcrossing process, conducted two to four times with the hybrids tested, chromosomes may be lost, or changes in chromosome pairings may result in changes in the expression of various traits. Therefore, plants that should be very similar may be quite different. Because we know nothing of the inheritance of resistance to *H. glycines* in *G. tomentella,* we do not know what to expect in the transfer of this resistance.

The variation in responses of the derived fertile lines from the *G. max* × *G. tomentella* cross to *H. glycines* may be because of several factors. Resistance to *H. glycines* appears to be multigenic (Caviness, 1992). During the backcrossing process, one or more major or minor genes for resistance may have been lost. In addition, one or more resistance genes from the wild perennial *Glycine* spp. may not function properly when transferred to the *G. max* genome.

The availability of soybean plants resistant to three races of *H. glycines* derived from an interspecific cross between *G. max* and *G. tomentella* suggests that genetic material has been transferred across species delimitations. The resistance in the hybrids had to result from the transfer of genetic material from *G. tomentella* to *G. max;* this would be the first report of genetic transfer via the sexual process of a trait from a wild perennial *Glycine* sp. to the cultivated soybean.

Literature Cited

Caldwell, B. E., C. A. Brim, and J. P. Ross. 1960. Inheritance of resistance to soybean cyst nematode, *Heterodera glycines.* Agronomy Journal 52:635–636.

Caviness, C. E. 1992. Breeding for resistance to soybean cyst nematode. Pp. 143–156 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of soybean cyst nematode. St. Paul, MN: APS Press.

Golden, A. M., J. M. Epps, R. D. Riggs, L. A. Duclos, J. A. Fox, and R. L. Bernard. 1970. Terminology and identity of infraspecific forms of the soybean cyst nematode (*Heterodera glycines*). Plant Disease Reporter 54: 544–546.

Johnson, A. W., and J. Feldmesser. 1987. Nematicides—a historical review. Pp. 448–454 *in* J. A. Veech and D. W. Dickson, eds. Vistas on nematology. Hyattsville, MD: Society of Nematologists, Inc.

Kollipara, K. P., R. J. Singh, and T. Hymowitz. 1997. Phylogenetic and genomic relationships in the genus *Glycine* Willd. Based on sequences from the ITS region of nuclear rDNA. Genome 40:57–68.

Noel, G. R. 1992. History, distribution and economics. Pp. 1–14 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of soybean cyst nematode. St. Paul, MN: APS Press.

Riggs, R. D., and D. P. Schmitt. 1988. Complete characterization of the race scheme for *Heterodera glycines.* Journal of Nematology 20:392–395.

Schmitt, D. P., F. T. Corbin, and L. A. Nelson. 1983. Population dynamics of *Heterodera glycines* and soybean response in soils treated with selected nematicides and herbicides. Journal of Nematology 15:432–437.

Schmitt, D. P., and G. Shannon. 1992. Differentiating soybean responses to *Heterodera glycines* races. Crop Science 32:275–277.

Singh, R. J., K. P. Kollipara, and T. Hymowitz. 1993. Backcross (BC2–BC4)—derived fertile plants from *Glycine max* and *G. tomentella* intersubgeneric hybrids. Crop Science 33:1002–1007.

Wrather, J. A., S. C. Anand, and S. R. Koenning. 1992. Management by cultural practices. Pp. 125–131 *in* R. D. Riggs and J. A. Wrather, eds. Biology and management of soybean cyst nematode. St. Paul, MN: APS Press.

Young, L. D., E. E. Hartwig, S. C. Anand, and D. Widdick. 1986. Response of soybeans and soybean cyst nematodes to cropping sequences. Plant Disease 70: 787–791.